

The Light Growth Response of *Phycomyces*

K. W. FOSTER and E. D. LIPSON

From the Division of Biology, California Institute of Technology, Pasadena, California 91109. Dr. Foster's present address is the Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80302.

ABSTRACT With the help of an automated tracking system we have studied the characteristics of the transient light growth response of *Phycomyces*. The response shows a sharply defined latency. The Q_{10} of the reciprocal latency is 2.4. Response patterns at different peaks of the action spectrum are the same. The gradual variation of response magnitude over a wide range of adapted intensities parallels that of phototropism. The responses to saturating stimuli exhibit a strong oscillation with a constant period of 1.6 min and variable damping. The growth responses to sinusoidally varying light intensities show a system bandwidth of 2.5×10^{-3} Hz. The linear dependence of phase shift on frequency is largely attributable to the latency observed with pulse stimuli. In the high intensity range a previously suspected increase of the steady-state growth rate with intensity has been confirmed. The light growth responses of mutants selected for diminished phototropism have been investigated. Many of these mutants have sizable but grossly distorted growth responses.

INTRODUCTION

We present in this paper a detailed study of the light growth response of the sporangiophore of *Phycomyces* as well as some results with mutants that were isolated for defective phototropism. These mutants illustrate a variety of ways in which this defective phenotype may arise from abnormal light growth responses. The light growth response is similar in character to the membrane potential responses of light sensors of more complex organisms. Since *Phycomyces* is amenable to genetic analysis (Cerdá-Olmedo, 1973, Ootaki et al., 1973) it may be possible to obtain a complete description of its functional organization. Such a description should include certain general features, such as adaptation, shared by sensory transducers of higher organisms.

The light growth response was discovered by Blaauw (1909) and described in detail by Blaauw (1909, 1914, 1915, 1918), Castle and Honeyman (1935), Castle (1966), Delbrück and Reichardt (1956), and others (Bergman et al., 1969). It is the preferred response for detailed quantitative analysis because the stimulus is easy to quantitate and the response preserves geometric symmetry.

The light responses have a range adjustment mechanism whereby the growth response depends on the intensity to which the specimen has been preadapted. The "level of adaptation," A , has been defined (Delbrück and Reichardt, 1956) as the intensity to which the specimen has been adapted for long times (about 40 min). The definition can be generalized to non-steady-state situations by defining the instantaneous level A as the virtual intensity to which it would have to be adapted to give the actual response characteristics. The response to a light pulse of duration Δt (short compared to the latency) and intensity I is a function of the quantity $S \equiv \Delta t(I - A)/A$, which we call the "subjective stimulus." The dimension of S is time, measured here in minutes. The kinetics of the level of adaptation are described approximately by the equation $dA/dt = (I - A)/b$, where b is the time constant of adaptation. Thus if a specimen is adapted to a certain level A and the light is turned off, the level of adaptation decays exponentially with a time constant b . At 20°C, b is approximately 5 min.

It is the purpose of this paper to summarize the response characteristics to pulse, step, and sine-wave stimuli and compare them to findings of others on analogous photostimulus response systems.

MATERIALS AND METHODS

Strains

The strains used in this report are listed in Table I. Mutagenesis and mutant selection procedures have been described elsewhere (Heisenberg and Cerdá-Olmedo, 1968; Bergman et al., 1973).

TABLE I
ORIGIN OF *PHYCOMYCES* STRAINS

Strain	Genotype	Origin
NRRL1555	(-)	From Northern Regional Research Laboratories (Peoria, Ill.)
UBC24	(-)	From Dr. R. G. Bandoni, Botany Department, University of British Columbia (Vancouver, B.C.)
C2	<i>carA5</i> (-)	From NRRL1555, by procedure 1
C21	<i>mad-7</i> (-)	From NRRL1555, by procedure 1
C47	<i>mad-35</i> (-)	From NRRL1555, by procedure 1
C63	<i>mad-54</i> (-)	From NRRL1555, by procedure 1
C68	<i>mad-59</i> (-)	From NRRL1555, by procedure 1
C106	<i>mad-97</i> (-)	From NRRL1555, by procedure 1
C110	<i>mad-102</i> (-)	From NRRL1555, by procedure 1
C149	<i>mad-120</i> (-)	From NRRL1555, by procedure 2
C150	<i>mad-121</i> (-)	From NRRL1555, by procedure 2
S5	<i>carA51 mad-202</i> (-)	From UBC24
S14	<i>carA53 mad-205</i> (-)	From UBC24
S18	<i>carA57 mad-209</i> (-)	From UBC24
S37	<i>carA76 mad-225</i> (-)	From UBC24

Culture Conditions

Sporangiophores were grown in shell vials (12-mm diameter x 35-mm high) containing potato dextrose agar medium filled to 10 mm from the top. To produce healthy sporangiophores an average of one to two viable spores were planted in each vial. The vials were incubated enclosed in glass jars in room light (about 20 $\mu\text{W}/\text{cm}^2$) until the first crop of sporangiophores appeared, then removed from the jars, and incubated in a light box with overhead illumination (4 $\mu\text{W}/\text{cm}^2$). The box was humidified to 60–80 % and maintained at a temperature of 22°C. The sporangiophores were plucked each evening so that a fresh crop would be ready the next morning. To minimize variability due to age of the specimens, we generally used only the second, third, and fourth crops. Specimens that were found to have growth rates less than 30 $\mu\text{m}/\text{min}$ were rejected.

Tracking Machine

A tracking machine, described in detail elsewhere (Foster, 1973), automatically records responses of *Phycomyces*. This machine maintains the spherical sporangium fixed in space to a precision of about 0.5 μm . It uses a servo-system that continually lowers the three-dimensional stage and moves it horizontally so as to keep the sporangium position fixed. Two orthogonal red-light beams in the horizontal plane are used to form two shadow images of the sporangium; *Phycomyces* is blind to this red tracking light (632.8 nm from a He-Ne laser). Three pairs of light detectors are used to compare the light intensity at opposite edges of the sporangium shadow image. The motor-driven stage is adjusted continuously to maintain the equality of the two intensities for each pair.

The sporangiophore is tracked in an enclosed chamber with a controlled environment. The temperature, unless otherwise indicated, is $20.5 \pm 0.5^\circ\text{C}$.

Light Sources and Stimulation

For experiments at relatively low adapted levels, a 150-W quartz-iodide-tungsten lamp (GE 1958, General Electric Company, Cleveland, Ohio) operated at 24 V was used. For "blue light" a 5-mm thick Corning 5-61 filter (Corning Glass Works, Corning, N. Y.) and a 5-mm thick Schott KG-1 heat filter (Fish-Schurman Corp., New Rochelle, N.Y.) were used. The former has a broad bandpass from 360 to 520, peaking at 460 nm (Bergman et al., 1969, sect. 38). To obtain monochromatic light at 380 nm or 455 nm, two interference filters (Thin Film Products Div., Infrared Industries, Inc., Waltham, Mass.) of 10-nm bandwidth were used.

For experiments at high intensities two argon-ion lasers were employed. An RCA Model LD 2108 (RCA Corp., Lancaster, Pa.) provided 4 mW of 488-nm light. A Coherent Radiation Model 52G (Coherent Radiation Laboratories, Palo Alto, Calif.) produced over 700 mW at 488 nm. The output intensity of the Coherent Radiation laser was programmable by a control voltage; this feature was used to obtain sinusoidally varying intensities.

High intensities were measured with a Hewlett Packard Radiant Flux Meter 8335A with a Model 8334A Radiant Flux Detector option 013 with T19 Supersil 1 optical

window (Hewlett-Packard Co., Palo Alto, Calif.). This instrument has a flat response ($\pm 3\%$) from 180 nm to 1300 nm, but is insensitive below $1 \mu\text{W}/\text{cm}^2$ incident energy. A more sensitive broad-range detector was also used, a Schottky-Barrier ultraviolet-enhanced photodiode (PIN-10-UV) (United Detector Technology Inc., Santa Monica, Calif.). Its current was measured with a Model 134 electrometer (Princeton Applied Research Corp., Princeton N. J.) in feedback mode.

Pulse and step stimuli were given with the aid of a set of quartz inconel neutral-density filters (0.3, 0.5, 1.0, 2.0, 4.0 OD) either individually or additively. A 0.15–4.0 OD inconel coated, neutral-density wedge A-6040 (Eastman Kodak Co., Rochester, N. Y.) was electronically controlled to provide versatile and automatic control of stimulus size.

Response Measurement

To obviate phototropic bending, bilateral oblique illumination (Delbrück and Reichardt, 1956) was used. In early experiments the beam from a single light source was directed to both sides of the sporangiophore via a beam splitter and pair of mirrors. In later experiments a mechanical beam alternator operating at 10 Hz was used in place of the beam splitter. In both cases the light beams were incident on the sporangiophore at 30°C to the horizontal.

The instantaneous position of the stage was measured by a set of three linear-variable-differential transformers (LVDT). Changes in the stage position corresponded precisely to growth of the sporangiophore. The vertical position signal was electronically differentiated yielding the vertical growth velocity dz/dt .

Data Analysis

The data output from the machine consisted of a strip-chart record of the vertical growth velocity. Each response was transferred by hand onto a sheet of tracing paper for easier comparison with other records. Response curves measured under identical conditions were averaged and are so displayed in the Results section.

The magnitude of the response has been quantitated using two criteria (Fig. 1). One measure (Oort, 1932) is the area R_A of the positive phase of the growth velocity above the basal growth rate before the response. The other measure we have used is the peak-to-peak amplitude R_P . This measure emphasizes the saturation of the growth rate for large stimuli but does not reflect increase in the *duration* of the response.

Latency and initial acceleration were determined from the best straight line through the 20 and 80 % points of the first rising slope of the response (Fig. 1). The latency was taken as the time between the start of the stimulus and the intersection of this straight line with the level of the basal growth rate. The slope of this line has been taken as the initial acceleration, α .

Both response measures R_P and R_A were analyzed as a function of the subjective stimulus $S \equiv \Delta t(I-A)/A$, in minutes. For each experimental condition the data were fit by nonlinear least squares (Hamilton, 1964) to the hyperbolic form

$$R = R_o \frac{S}{S + S_o}.$$

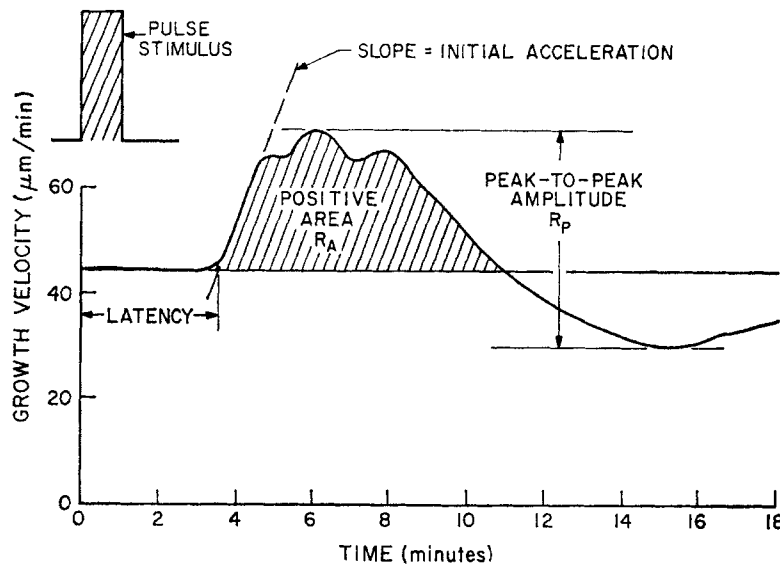


FIGURE 1. A typical light growth response of *Phycomyces*. The parameters used in analyzing such responses are defined on the figure.

R_o is the saturation value of R and S_o is the value of S for which $R = R_o/2$. We have plotted our results with $\log_{10} S$ as the abscissa. Then the curve takes on a symmetrical sigmoidal shape according to

$$R = \frac{R_o}{2} \{1 + \tanh [1.151 (\log_{10} S - \log_{10} S_o)]\}.$$

For each S the values of R_P and R_A were measured from an averaged trace of several responses. In the least-squares analysis the R values were weighted statistically in proportion to the number of observations that constituted each average curve. Note that the errors assigned to estimates of the parameters R_o and S_o are standard errors based on the quality of the fit.

RESULTS

Temperature Dependence of Response

Fig. 2 shows typical light growth responses at various temperatures to an 0.5-min stimulus at 100 times the adapting intensity, in the "normal" range as defined by Delbrück and Reichardt (1956). These are single responses taken from experiments on two specimens, to show clearly the shape of each response. The adapted level was about $0.2 \mu\text{W}/\text{cm}^2$. The stimulus was $20 \mu\text{W}/\text{cm}^2$ for an 0.5-min duration ($\log_{10} S = 1.7$). Temperature was controlled by a thermoelectric element and was varied from 15 to 25°C . With a decrease in temperature of 10°C the latency lengthens from 2.5 min to 6 min without much change in the shape of the response. The total area of the re-

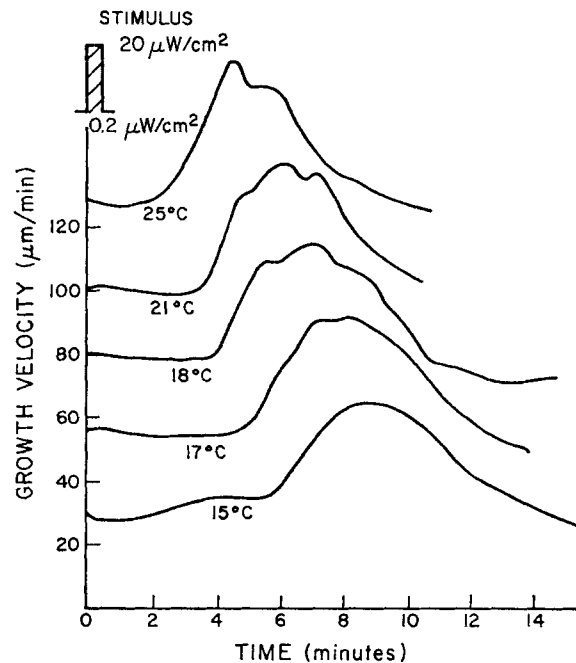


FIGURE 2. Temperature dependence of responses to a 0.5-min pulse of magnitude $\log_{10} S = 1.7$ using broad blue light. $A = 0.20 \mu\text{W}/\text{cm}^2$. The curves are displaced $20 \mu\text{m}/\text{min}$ along the growth velocity scale.

sponse does not seem to be affected at these temperatures. This result is similar to that found by Charlton and Naka (1970) for the catfish S potential. The magnitude of this temperature dependence indicates that the duration of the latency is probably governed by enzymatic reactions.

Responses to Pulse Stimuli of Various Durations and Colors and at Different Levels of Adaptation

Responses were measured to stimuli under diverse conditions. Some of these responses are illustrated in Figs. 3 and 4. Three sets of response magnitudes are analyzed in Fig. 5.¹ Consider first the responses to various stimuli as shown in Fig. 3 *a*. Here the responses are plotted for individual 0.5 min pulses of 455-nm light with magnitudes from $\log_{10} S = 0.03$ to 3.5. The adapted level was $40 \text{ pW}/\text{cm}^2$. Each response has been displaced along the ordinate so that the basal growth rate of each response is placed at the position of the $\log_{10} S$ corresponding to the given stimulus. Responses to 380-nm light stimuli are illustrated in Fig. 3 *b*. The adapted light level was $180 \text{ pW}/\text{cm}^2$.

Responses to broad-blue light (360–520 nm) were measured for 0.5 min

¹ Response curves for the other stimuli mentioned in the text (summarized in Table II) are available on request from either author. Corresponding analysis curves for each set are also available.

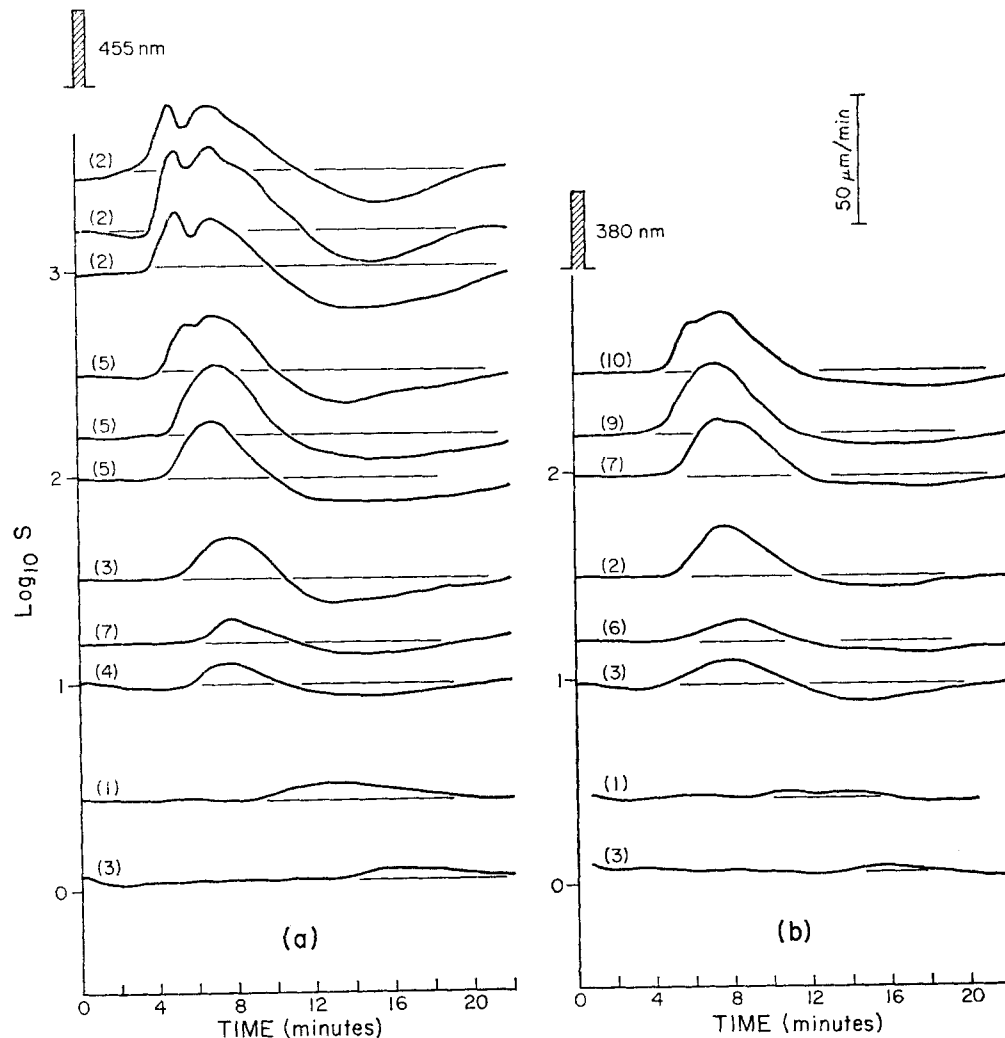


FIGURE 3. Growth response of the albino mutant C2 to 0.5 min light pulses at two wavelengths: (a) 455 nm with $A = 40 \text{ pW/cm}^2$ and (b) 380 nm with $A = 140 \text{ pW/cm}^2$. For each response the basal growth rate level is indicated by a horizontal line. The curves are displaced such that this level intersects the ordinate at the appropriate level of $\log_{10} S$, where $S \equiv \Delta t (I - A)/A$ is the "subjective stimulus" in minutes. The vertical scale for growth velocity is indicated at the upper right of the figure. Note that the zero level for each response is not displayed on the figure; basal growth rates were typically $50 \text{ } \mu\text{m/min}$. The number of responses averaged to produce each curve is indicated in parentheses.

(Fig. 4 a), as well as 1-, 2-, and 5-min stimuli with an adapted level of 400 pW/cm^2 . When comparing figures note that on the ordinate in each case equal S corresponds essentially to an equal number of photons in the stimulus relative to the adapted level. Responses at a much higher adapted level, 2.5

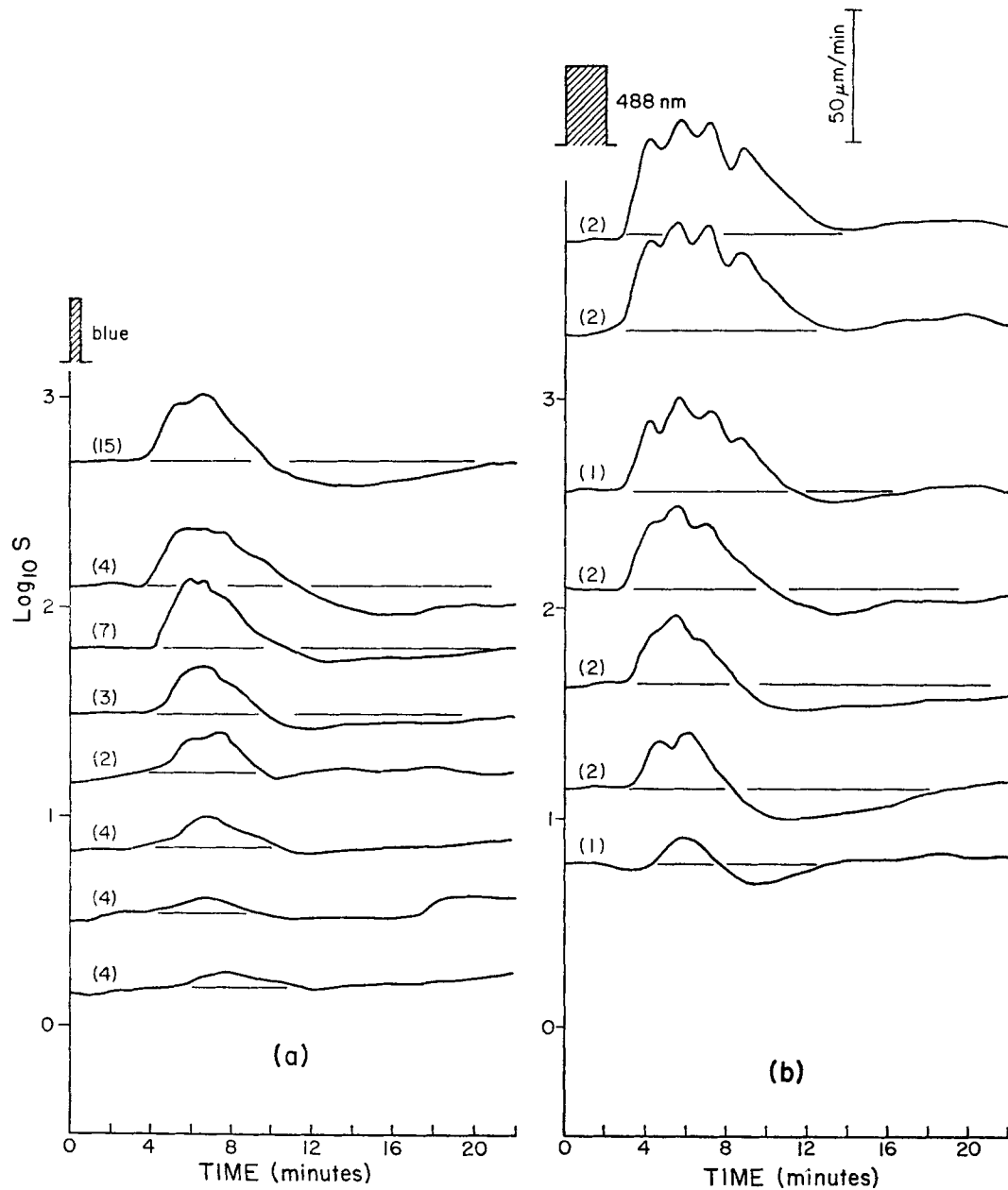


FIGURE 4. (a) Growth responses of C2 to broad blue light pulses of 0.5-min duration with $A = 400 \text{ pW/cm}^2$. (b) Growth responses of C2 to light pulses of 2-min duration at 488 nm with $A = 2.5 \text{ } \mu\text{W/cm}^2$. See caption to Fig. 3.

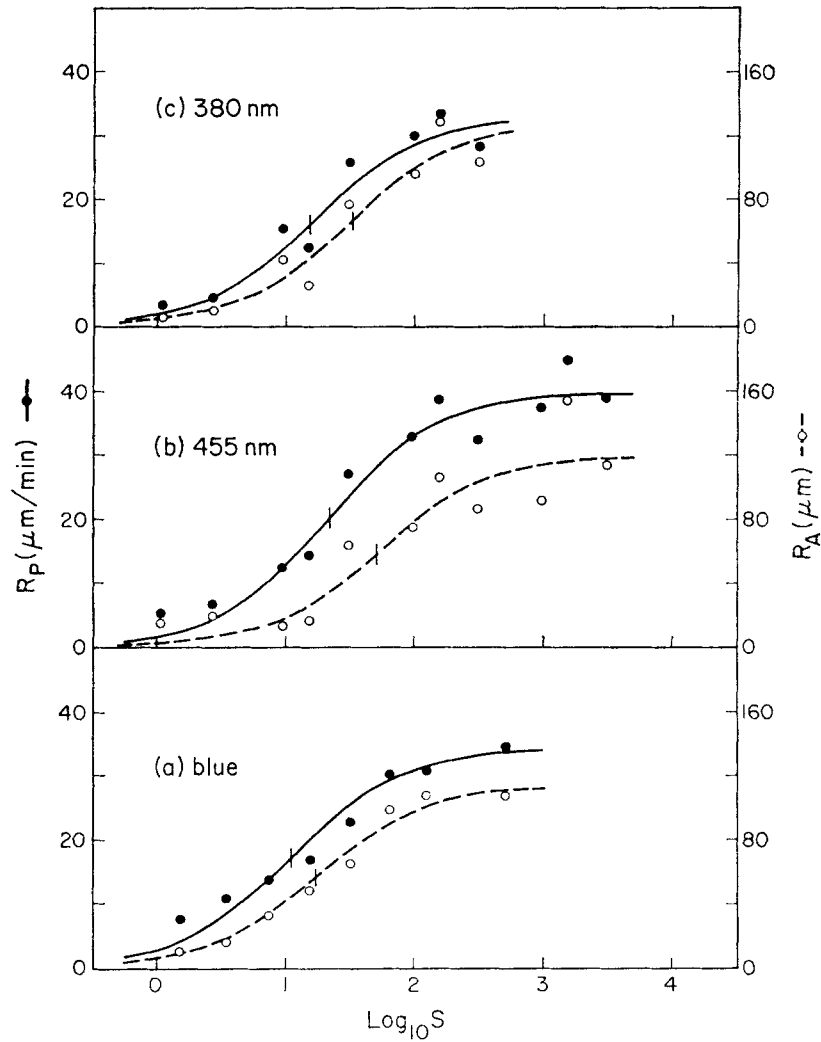


FIGURE 5. Analysis of response magnitude as a function of stimulus size for different wavelengths (a) broad blue with $A = 400 \text{ pW/cm}^2$ (from Fig. 4 a), (b) 455 nm with $A = 40 \text{ pW/cm}^2$ (from Fig. 3 a), and (c) 380 nm with $A = 140 \text{ pW/cm}^2$ (from Fig. 3 b). In all cases the stimulus pulse width was constant at $\Delta t = 0.5 \text{ min}$. The logarithmic abscissa is defined by $S = \Delta t (I - A)/A$ measured in minutes. In each plot the solid circles and the solid curve correspond to the peak-to-peak amplitude R_P of the response; the open circles and the dashed curve correspond to the area R_A of the positive phase of the response. The curves are weighted nonlinear least-squares fits to the functional form $R = R_\infty S/(S + S_0)$.

$\mu\text{W}/\text{cm}^2$, were measured for 488-nm laser light with pulse durations of 0.5, 1, and 2 min (Fig. 4 b).

Wavelength Dependence of the Response

The action spectra of *Phycomyces* (Delbrück and Shropshire, 1960; Curry and Gruen, 1959) have four broad peaks at 280 nm, 375 nm, 450 nm, and 483 nm. The different peaks of sensitivity might correspond to more than one pigment. To investigate whether different wavelengths could produce different responses, three different colors of light stimuli were used, namely 380 nm, 455 nm, and "broad-blue" (360–520 nm).

All responses have been evaluated according to the criteria R_A and R_P (see Data Analysis section) and are summarized in Table II. The stimulus-

TABLE II
PARAMETERS FROM STIMULUS-RESPONSE CURVES

Wavelength	Adapted intensity A	Pulse duration Δt	R_{P_0}	S_{P_0}	R_{A_0}	S_{A_0}	S_{A_0}/S_{P_0}
nm	W/cm^2	min	$\mu\text{m}/\text{min}$	min	μm	min	
Blue*	4×10^{-10}	0.5	34 ± 1	11 ± 2	114 ± 4	17 ± 3	1.6 ± 0.4
455	4×10^{-11}	0.5	40 ± 2	22 ± 5	119 ± 10	52 ± 20	2.4 ± 1.0
380	1.4×10^{-10}	0.5	33 ± 2	15 ± 6	129 ± 14	30 ± 14	2.0 ± 1.2
Blue*	4×10^{-10}	1.0	40 ± 2	10 ± 2	100 ± 4	20 ± 3	2.0 ± 0.6
Blue*	4×10^{-10}	2.0	37 ± 3	17 ± 6	131 ± 11	46 ± 16	2.7 ± 1.3
Blue*	4×10^{-10}	5.0	39 ± 2	22 ± 7	159 ± 16	58 ± 27	2.6 ± 1.5
488	2.5×10^{-6}	0.5	38 ± 3	3 ± 2	99 ± 30	5 ± 7	1.6 ± 2.3
488	2.5×10^{-6}	1.0	33 ± 1	1 ± 1	115 ± 11	8 ± 5	8.5 ± 6.3
488	2.5×10^{-6}	2.0	42 ± 1	5 ± 1	255 ± 16	90 ± 26	16.7 ± 5.8

* Standard broad blue spectrum peaking at approximately 450 nm.

response curves for broad-blue, 380- and 455-nm light (from response data of Figs. 3 and 4 a) are compared in Fig. 5. Both R_A and R_P are plotted with the fits to the equation $R = R_0 S / (S + S_0)$. The curves fit the points well in most cases. Neither the plots of R_A nor those of R_P differ significantly for the different colors of the stimulating light used. This result does not preclude the possibility of several pigments being present, with absorption by these pigments feeding at an early stage into a common output path.

Effect of Pulse Duration

Consider the broad-blue (360–520 nm) data sets summarized in Table II. The peak-to-peak amplitude, R_P , is given for each of the stimulus durations of 0.5, 1, 2, and 5 min. In each case the stimuli were given from an adapted level of $400 \text{ pW}/\text{cm}^2$. R_{P_0} is approximately independent of pulse duration averaging $(36 \pm 1) \mu\text{m}/\text{min}$, while R_{A_0} increases with pulse durations longer

than 1 min. As might be expected, both the initial acceleration and latency are not influenced by the later part of stimuli when the duration of the stimulus becomes comparable to the latency and width of response. There is also an increase of both S_{P_0} and S_{A_0} (the stimulus size required to give half-maximum responses) for the 2- and 5-min stimuli relative to the 0.5- and 1-min stimuli. A similar observation may be made about the initial accelerations as shown in Fig. 6. The values of S_{A_0} for the acceleration increase roughly in proportion to the pulse duration, because after the initial part of the stimulus any additional pulse width has little effect on increasing the initial acceleration. At these pulse durations the stimulus for initial acceleration of response might better be described by $S'_A = (I - A)/A$, without the pulse width Δt . S'_{A_0} is nearly constant at 27 ± 11 . A similar value, $S'_{A_0} = 25 \pm 5$, was obtained for step responses.

The responses have also been studied at relatively high adapted-light levels of $2.5 \mu\text{W}/\text{cm}^2$ using 488-nm light (see example in Fig. 4 *b*). Table II gives response values for 0.5-, 1-, and 2-min stimuli for both criteria of response R_A and R_P . Here there are large differences between the two measures of the response. At higher stimuli R_P saturates, but R_A keeps rising because the duration of the positive response continues to increase. S_{A_0} is much greater than S_{P_0} (see Table II). This effect of the saturation of the maximum growth rate before the saturation of the response width is also observed at low adapted intensities, but to a lesser extent. If the data for blue light, 455 nm and 380 nm are grouped together, the ratio of S_{A_0} to S_{P_0} is 1.9 ± 0.3 .

Effects of Level of Adaptation

Fig. 7 *a* shows how the growth response to a fixed subjective stimulus changes as a function of the level of adaptation. Apart from the longer latency at the lowest adaptation level, the responses are similar. Similarly, Fig. 7 *b* shows typical responses of the same specimen at various levels of adaptation to *step* stimuli with $I/A = 10$. These also are similar except for latency.

If the magnitude of the response as a function of the adapted level is examined over the whole range of 10^9 , a broad peak is seen. For $\log_{10} S = 2$, we have plotted from all available data the dependence of R_P as a function of A (Fig. 8). It is useful to compare this curve to data for the phototropic rate of Reichardt and Varjú (1958) and Bergman et al. (1969). We have normalized their peak troping rates to the same value. Both at low and at high intensities the phototropic bending rate is reduced in a manner similar to the growth response.

The response R_P to stimuli of $\log_{10} S = 2$ varies little over the million-fold range of A between $10 \mu\text{W}/\text{cm}^2$ where "bleaching" is believed to overtake pigment regeneration and A_D , the threshold for a growth response. A_D is the intensity, about $8 \text{ pW}/\text{cm}^2$ (455 nm), at which any further lowering of

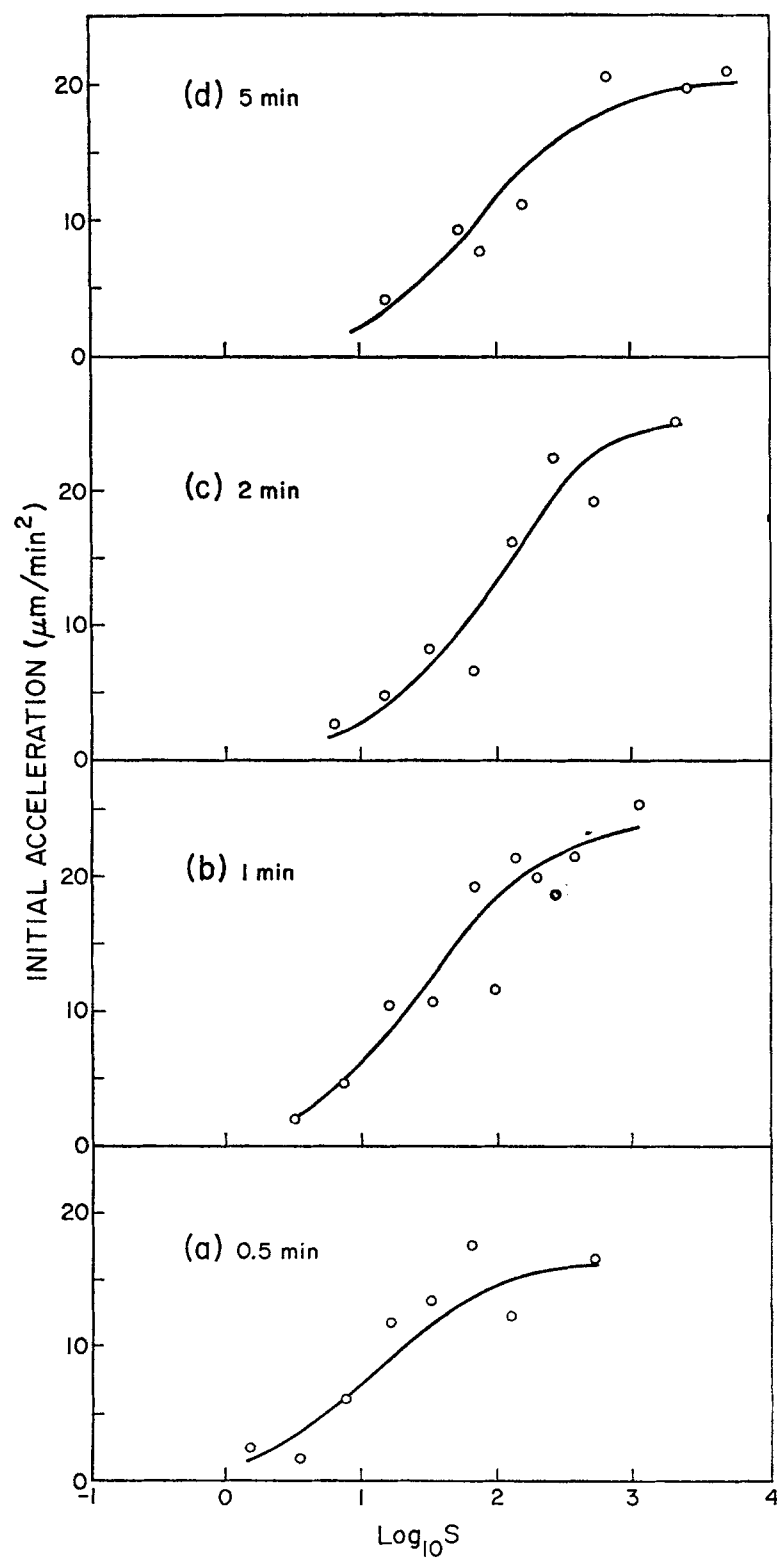


FIGURE 6. Analysis of initial acceleration α as a function of stimulus size for different pulse durations of broad blue light with $A = 400 \text{ pW}/\text{cm}^2$. The curves are least-squares fits to the function $\alpha = \alpha_0 S / (S + S_{a0})$.

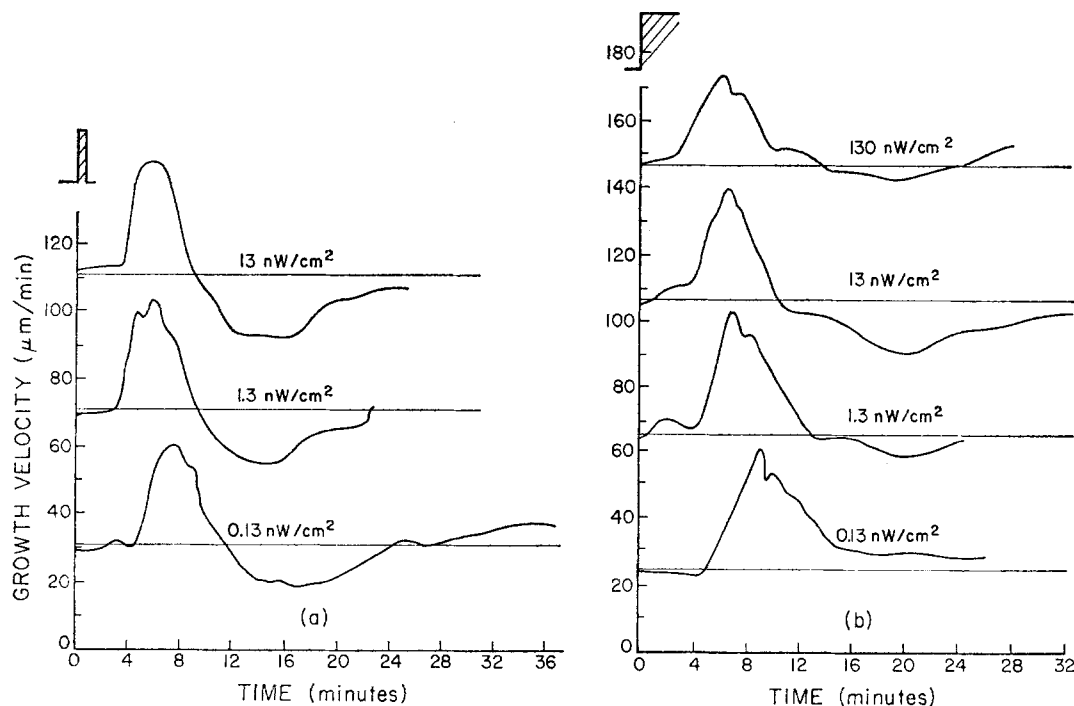


FIGURE 7. (a) Growth responses to pulse stimuli at different levels of adaptation. Single responses of one specimen to 0.5-min pulse stimuli with $\log_{10} S = 1.7$. The adapted level is indicated for each curve. Each curve is displaced by $40 \mu\text{m}/\text{min}$ and no curve has been drawn at its absolute growth rate. (b) Growth responses at different levels of adaptation to step stimuli of $\log_{10} (I/A) = 1$. Same specimen as in (a).

the adapting intensity produces no significant increase in the responses to a stimulus of some fixed absolute intensity. This threshold for the growth response probably corresponds to the "dark light" of human vision research (Rushton, 1961).

Responses to Step Changes of Light Intensity

Responses to step changes of light intensity have not been extensively investigated here. Fig. 9a shows the response to a step in intensity from $0.4 \text{ nW}/\text{cm}^2$ to $400 \text{ nW}/\text{cm}^2$ (blue light). The response, including an undershoot, is quite similar to the response to pulse stimuli. However at high adapted intensities a step from $2.5 \mu\text{W}/\text{cm}^2$ to $32 \mu\text{W}/\text{cm}^2$ (Fig. 9b) produces an extremely long response that seems never to return to the basal rate.

Responses to Steps and Pulses Down in Intensity (Dark Growth Responses)

Responses to negative stimuli are generally small. Fig. 9c shows the response to a 0.5-min pulse down from $400 \text{ nW}/\text{cm}^2$ to $0.4 \text{ nW}/\text{cm}^2$. This response

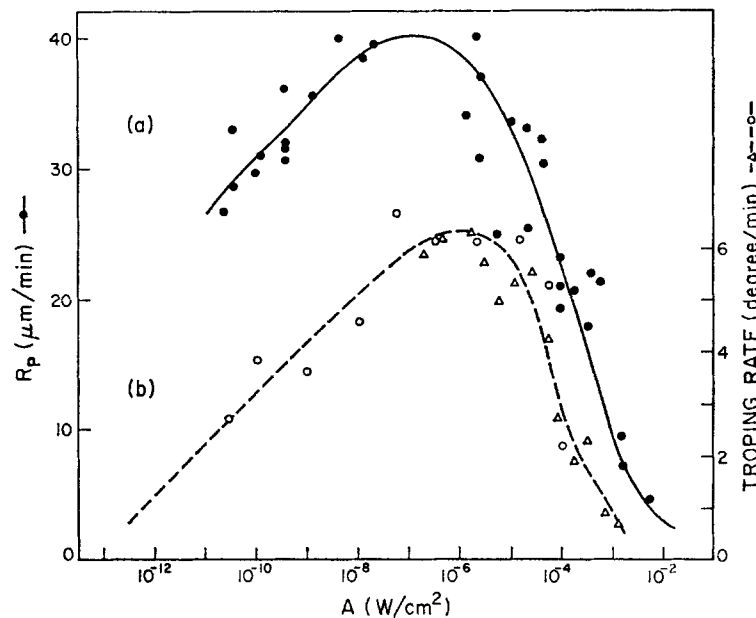


FIGURE 8. (a) Response amplitude R_P (peak-to-peak) as a function of A for fixed stimulus size $\log_{10} S = 2.0$. (b) Phototropic bending rate as a function of A . The open triangles are from Bergman et al. (1969). The open circles are from Reichardt and Varjú (1958). The latter have been renormalized (reduced 10%) to match with the former.

may be compared to the 0.5-min pulse up ($0.4 \text{ nW}/\text{cm}^2$ to $400 \text{ nW}/\text{cm}^2$) of magnitude $\log_{10} S = 2.7$ shown at the top of Fig. 4 *a*. It is clearly very much smaller in magnitude indicating a strong nonlinearity (rectification) of the system. Fig. 9 *d* shows a response to a step down from $400 \text{ nW}/\text{cm}^2$ to $0.4 \text{ nW}/\text{cm}^2$ and Fig. 9 *e* a step down from $7.2 \text{ mW}/\text{cm}^2$ to $0.11 \text{ mW}/\text{cm}^2$. Just as with positive steps, the response is much more prolonged at high intensities than at moderate intensities and after transients have died away does not return to the same basal rate. In comparing Fig. 9 *a* to Fig. 9 *d* note that the latencies are approximately equal for steps up as for steps down.

Latency

The present data show very clearly that the response starts suddenly after a latent period of a few minutes. The latencies for the 455-nm responses to pulses shown in Fig. 3 *a* are plotted in Fig. 10 *a* as a function of

$$1/\log_{10} ([I - A]/A).$$

The data are fit fairly well (empirically) by a straight line extrapolating to 2.3 min for $I \rightarrow \infty$. In Fig. 10(b) $1/(\text{latency} - 2.3)$ is plotted versus

$$\log_{10} ([I - A]/A).$$

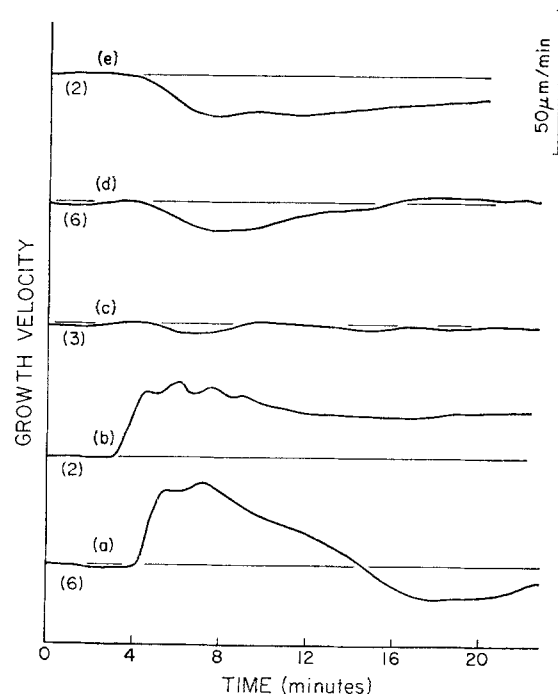


FIGURE 9. Responses to steps and pulses under various conditions. (a) Step up from $A = 0.4 \text{ nW/cm}^2$ to $I = 400 \text{ nW/cm}^2$ using broad blue light, (b) step up from $A = 2.5 \text{ μW/cm}^2$ to $I = 320 \text{ μW/cm}^2$ using 488 nm, (c) 0.5-min pulse down from $A = 400 \text{ nW/cm}^2$ to $I = 0.4 \text{ nW/cm}^2$ using broad blue, (d) step down from $A = 400 \text{ nW/cm}^2$ to $I = 0.4 \text{ nW/cm}^2$ using broad blue, (e) step down from 7.2 mW/cm^2 to 0.11 μW/cm^2 using 488 nm.

These results are compared to the work of Castle (1932) (dashed lines) for the dark growth response. Human subjects also show a similar dependence of latency in response to flashes of light when pre-dark-adapted (Bartlett and Macleod, 1954).

For stimuli at adapted levels above threshold, the results of Castle (1930, 1932) and Castle and Honeyman (1935) were confirmed. Latency decreases very gradually with the stimulus size. Like Castle we found that it is the stimulus size S which determines latency provided the stimulus duration is less than 1 min. The latency decreases with increasing intensity.

Fine Structure of Response

One noteworthy feature of responses saturated with respect to the growth rate is the multiple peaks and valleys that appear (see Figs. 3 and 4 and Oort [1932]). This fine structure is evidently associated with the growth-regulating part of the transducer chain rather than with the initial light-sensing system; the responses to a very different type of stimulus, namely placing a 1.5-cm

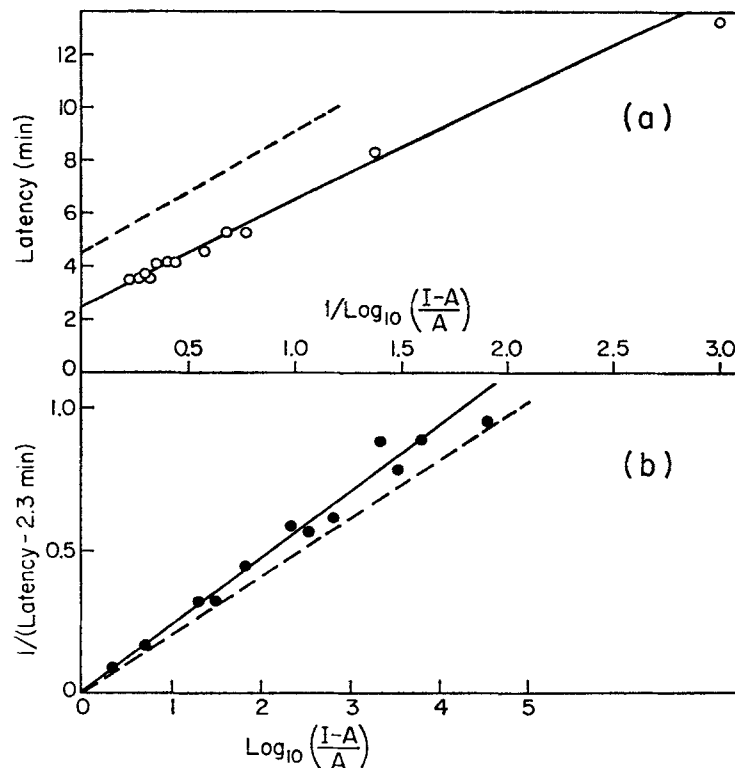


FIGURE 10. Latencies of growth responses. (a) The solid line is the latency as a function of $1/\log_{10} [(I - A)/A]$, where $A = 40 \text{ pW/cm}^2$ (455 nm) and I the intensity of each 30-s duration stimulus (data from Fig. 3 a). The dashed line is taken from the dark growth response latencies measured by Castle (1932). (b) Replot of data of the solid line of (a) as $(\text{latency} - 2.3 \text{ min})^{-1}$ as a function of $\log_{10} [(I - A)/A]$. The dashed curve is taken from the dark growth response latencies measured by Castle (1932). The ordinate for the dashed line has been changed to $(\text{latency} - 4.5 \text{ min})^{-1}$.

square glass house (symmetrical “avoidance” stimulus) around the specimen, produces responses with similar fine structure (Foster, 1972). By monitoring the horizontal coordinates, we have checked that during this “ringing” there are no significant tropic oscillations. Fig. 11 shows a particularly noiseless response to a step change (at 488 nm) from $80 \text{ } \mu\text{W/cm}^2$ to $5100 \text{ } \mu\text{W/cm}^2$. Nine cycles are clearly visible with a mean period of $1.6 \pm 0.1 \text{ min}$. Their amplitude decreases exponentially (Fig. 11 b) with a time constant of $7.2 \pm 0.4 \text{ min}$.

If we assume this behavior arises from a second-order system with a transfer function of the form $1/([s^2/\omega_o^2] + [2\zeta s/\omega_o] + 1)$ (where s is the Laplace transform operator) then we find a “dampening ratio,” $\zeta = (0.035 \pm 0.002)$ and an “undamped natural frequency” of $f_o = \omega_o/2\pi = (11 \pm 1) \times 10^{-3} \text{ Hz}$ ($0.66 \pm 0.06 \text{ min}^{-1}$). What “feedback” or “low-pass filter”

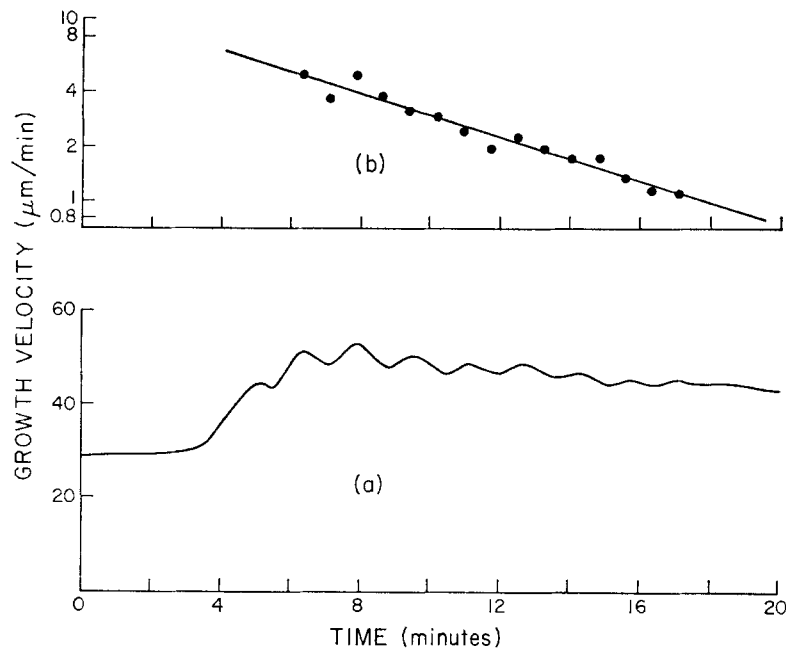


FIGURE 11. (a) Fine structure exhibited on a single response to a step from $A = 80 \mu\text{W}/\text{cm}^2$ to $I = 5100 \mu\text{W}/\text{cm}^2$ using 488 nm light. Period of oscillations $T_o = (1.6 \pm 0.1)$ min. (b) Exponential decay of the peak-to-peak amplitude of the oscillations with a time constant $\tau = (7.2 \pm 0.4)$ min.

type mechanisms are involved in producing this transfer function are unknown. For a review of biochemical mechanisms which produce oscillations see Hess and Boiteux (1971).

Sine Wave Stimuli

To examine the nonlinear character of the system, we employed sinusoidally varying input intensities. The intensity (at 488 nm) was swept sinusoidally over a range from $0.03 \mu\text{W}/\text{cm}^2$ to $3 \mu\text{W}/\text{cm}^2$. In Fig. 12 several examples of responses are shown. Over a narrow frequency range from 0.001 Hz to 0.004 Hz the responses look relatively sinusoidal as would be expected for a linear system. The apparent frequency dependence of the phase shift is attributable to a constant time delay of 9.4 ± 0.3 min. This time delay corresponds to the time from the beginning of the stimulus to the maximum amplitude of response. The data also indicate that the phase of the response leads the input stimulus by about 100° (Fig. 13 b), i.e., the system responds to the rising phase of the sine-wave input. The response is 180° out of phase for a stimulus of an 11-min period. At low frequencies (<0.001 Hz) considerable rectification of the response is observed. In Fig. 13 a we have plotted the peak-to-peak response amplitude as a function of the stimulus frequency. A rapid rolloff is observed beyond 2.5×10^{-3} Hz (0.15 min^{-1}).

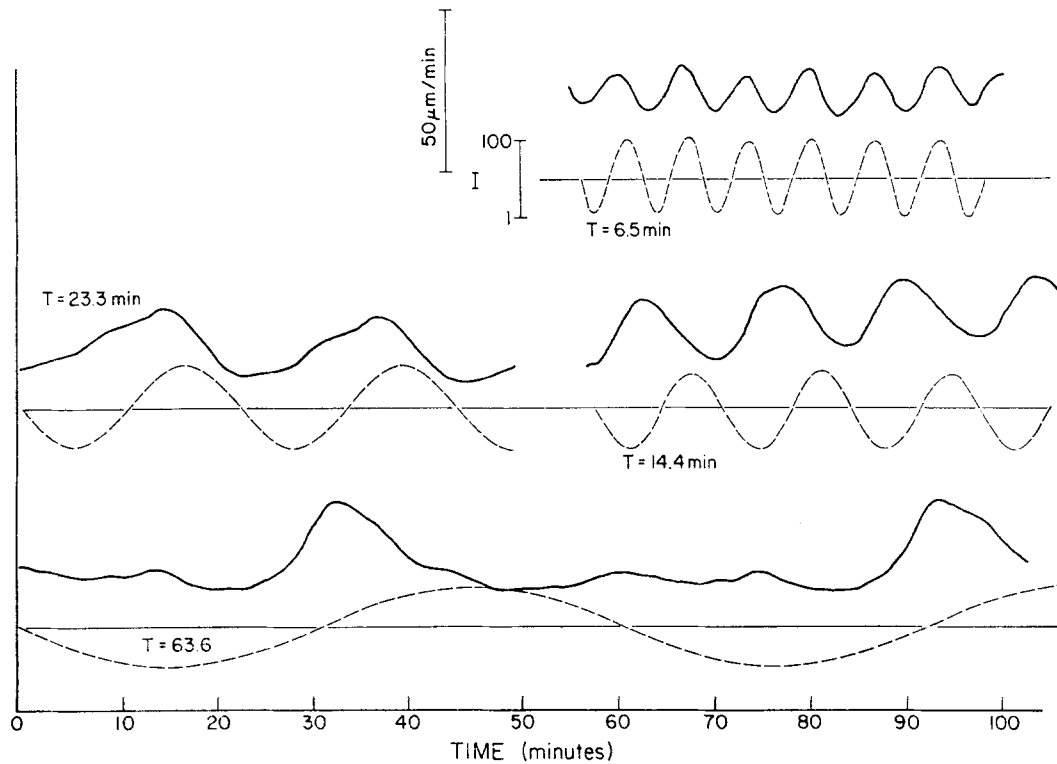


FIGURE 12. Growth velocity (solid curves) in response to sinusoidally varying light intensity (dashed curves). The periods are indicated with each set. For all sinusoids the minimum intensity was $0.03 \mu\text{W}/\text{cm}^2$ and the maximum was $3 \mu\text{W}/\text{cm}^2$.

Comparison of responses at a fixed frequency of $2.5 \times 10^{-3} \text{ Hz}$ for different adapted levels shows peak-to-peak amplitude changes similar to that for the responses to pulses shown in Fig. 7 a. At higher intensities the phase is shifted monotonically to earlier times indicating a reduction of latency even at the highest adapted intensities.

Growth Rate Increase at Very High Intensities

As noted earlier, the growth rate after a large step in the high intensity range does not return to the low-intensity growth rate. This phenomenon was first reported by Shropshire (Bergman et al., 1969) for a step up from $0.12 \mu\text{W}/\text{cm}^2$ to $5 \text{ mW}/\text{cm}^2$. Our results confirm this finding. Shropshire reported that if the intensity is raised more slowly to this high intensity then normal growth is resumed after the stimulus. We find however that if one waits for the long undershoot to diminish (sometimes as long as 90 min) then the higher growth rate is maintained. Lowering of the intensity thereafter brings the growth rate back to normal. Even very small steps produced this increased steady-state growth rate in the high intensity range (Fig. 14).

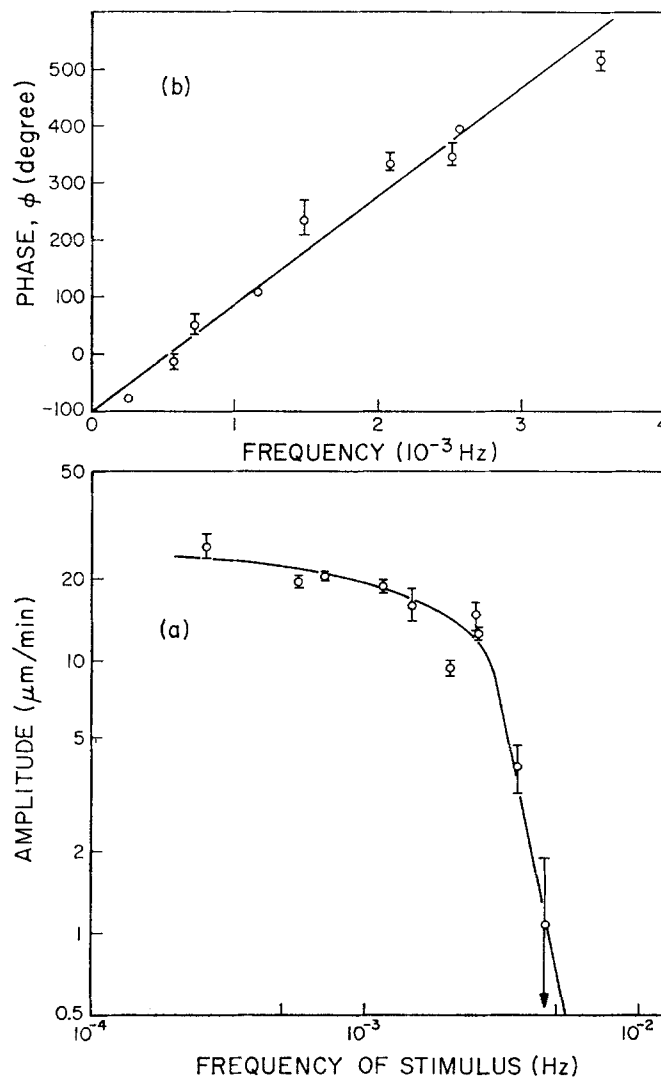


FIGURE 13. (a) Peak-to-peak amplitude of the growth velocity as a function of frequency of the light intensity (see Fig. 12). (b) Phase shift (as defined by text) as a function of frequency. The straight line is a weighted least-squares fit.

The increased growth occurs in the intensity range in which the size of growth and phototropic responses diminish. The data have been fitted to the equation $R = R_0 S / (S + S_0)$. We found this increased growth was "half-maximal" at a light intensity of $1.4 \pm 0.4 \text{ mW}/\text{cm}^2$ (488 nm). One may surmise that at this intensity the greater portion of the receptors are converted into non-functional photoproduct (bleached) and inhibitory control of the growth rate is removed.

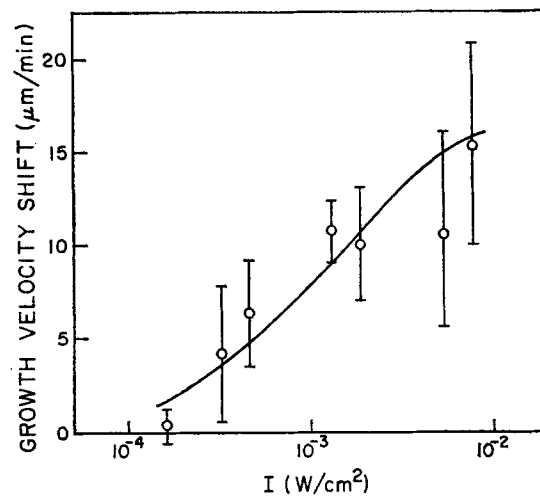


FIGURE 14. Shift in the steady-state growth rate as a function of absolute intensity.

Phenotypic Classes of Mutants with Aberrant Growth Responses

Bergman (1972 *a*) has studied a group of *mad* mutants (those with abnormal tropic responses to light). He divided this group into three classes: class 1-1 mutants, in which the abnormality affects both the light-controlled sporangiophore initiation (Bergman, 1972 *b*) and the phototropism, but which are normal for avoidance of barriers; class 1-2, which are abnormal only for phototropism; and class 2, which respond normally to light with sporangiophore initiation. No mutant has been found which is absolutely blind.

Three classes of mutants have been studied here. C47, a class 1-1 mutant, as shown in Fig. 15, shows a normal growth response at high intensities, but the threshold is raised by a factor 6×10^4 . It shows failure to respond at the same high intensities as wild type. C21 is sensitive over the same range as C47, however, it has a much delayed response (Fig. 16). Several phototropic effects result from this abnormality. One is that C21 exhibits rapid phototropic hunting (Bergman et al., 1969) with approximately one-third of the wild-type hunting wavelength (the length of the "frozen" wave in the stalk as a result of troping from side to side). Another is that it has a much larger aiming error (clockwise deviation of cell troping orientation as viewed from above relative to the direction of the light source). With 280-nm light (to which *Phycomyces* is negatively phototropic) this aiming error is sufficient to turn the troping direction 180° back toward the source. This occurs when the 280-nm intensity is near the threshold of C21 at a geotropic-phototropic equilibrium angle of 30° from the vertical.

A number of mutants (S37, S14, S18, S5) have been examined which were derived from the wild-type strain UBC24 and had become both photo-mutants of the class 1-2 and albinos (*carA*) in a single exposure to nitroso-guanidine. These mutants show long refractory periods analogous to those described by Oort (1932) and attributed by Delbrück and Reichardt (1956) to slow dark adaptation. In a sequence of three large stimuli separated by 45 min, the second stimulus gave no response. Oort describes this phenomenon for his wild-type strain when stimuli are separated by a much shorter time. Under these conditions our wild-type strains (NRRL1555 and UBC24) do not show a refractory period. This phenomenon bears further investigation.

Six class 2 mutants (C63, C68, C106, C110, C149, C150) have been examined (Figs. 17 and 18). These mutants seem to be quite similar and are presumably affected in their output apparatus. In all cases their growth response has abnormal shape. They share the common characteristics of a prolonged growth response with normal latency and weak phototropism to blue light, while having apparent phototropic thresholds for 280-nm light within a factor of 10 of that for wild type. They also trope continuously, although very slowly ($0.5^\circ/\text{min}$ for C149), even at moderate intensities ($2 \mu\text{W}/\text{cm}^2$ of 280 nm). This should be compared to bending rates of $6\text{--}20^\circ/\text{min}$ for C2 under similar conditions. Although they share similarities, they are not identical as their characteristic responses show. For example, unlike the others, C110 twists abnormally slowly. It may simply mature more slowly as it grows. In any case, its twisting rate reaches only about $4^\circ/\text{min}$ after 6 cm of growth. This mutant, unlike the others, shows a slight bending to unilateral blue light over a wide range of intensities (Bergman, 1972 *a*). As a class these mutants show poor phototropism in spite of the fact that they do have quite large growth responses which saturate at a lower growth rate and are longer in duration than that of wild type. It might be wondered why then after a number of hours they do not show more bending in the phototropic-geotropic equilibrium experiments. We believe that this occurs because of the response saturation at a small increase in growth rate and because the extended response is spread around the cell as a consequence of twisting of the cell during the response. This results in little differential growth rate of one side over the other. The potential usefulness of these mutants to dissect the stimulus-response pathway is apparent.

DISCUSSION AND SUMMARY

The *Phycomyces* light growth response system may be considered from the engineering viewpoint as a "black box." As such it may be described by a transfer functional giving the relationship between the input function (stimulus) and the output function (response). Mutations along this pathway

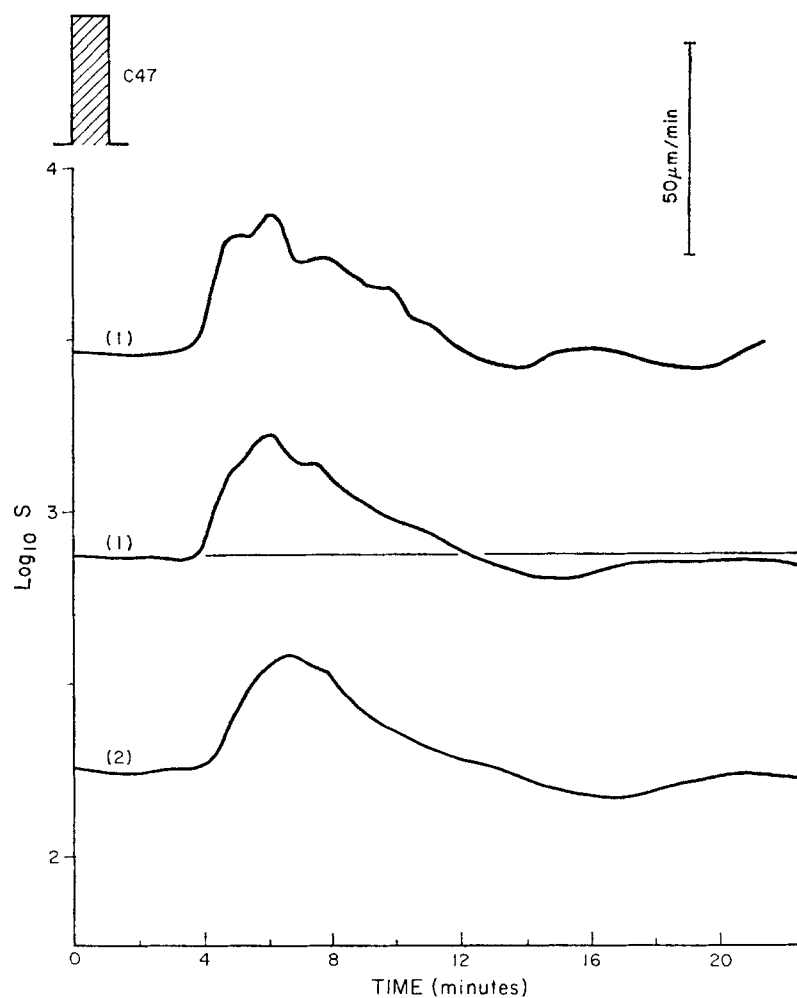


FIGURE 15. Responses of the photomutant C47 (class 1-1) to 1-min pulses from $A = 2.5 \mu\text{W}/\text{cm}^2$.

modify the functional in a manner analogous to destroying a component at random in an electronic black box.

The response of wild type was investigated in the time domain with pulse and step stimuli and in the frequency domain with sinusoids. With this information we can summarize many of the characteristics of the system. The light growth response had a bandwidth of $2.5 \times 10^{-3} \text{ Hz}$ (Fig. 13 *a*). At frequencies below 10^{-3} Hz (0.06 min^{-1}) the nonlinear property of rectification is apparent (Fig. 12). Comparison of increasing and decreasing pulses and steps of light also show considerable rectification (Figs. 9 and 4 *a*).

One widely observed characteristic of stimulus-response systems is the de-

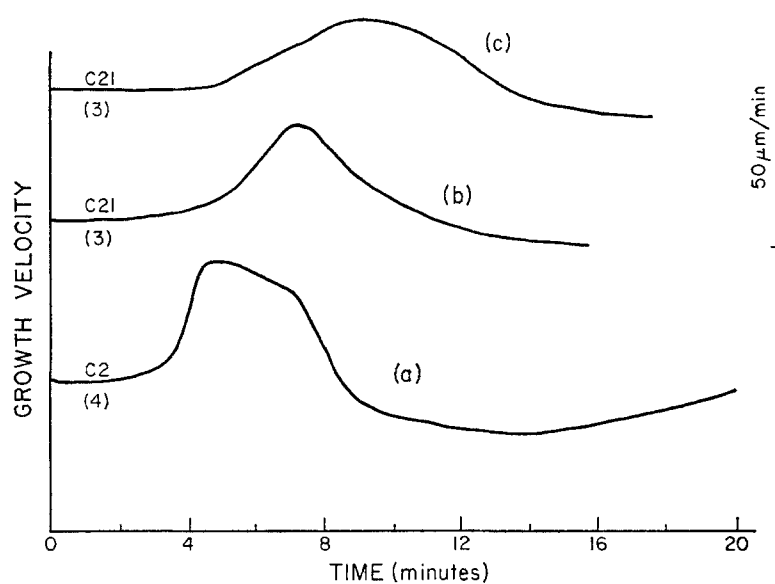


FIGURE 16. Comparison of the responses of C2 to the photomutant C21. (a) 0.5-min pulse of magnitude $\log_{10} S = 1.2$ from $A = 2.5 \mu\text{W}/\text{cm}^2$ for C2, (b) same pulse stimulus to C21, (c) step from $0.5 \mu\text{W}/\text{cm}^2$ to $8 \mu\text{W}/\text{cm}^2$ on C21.

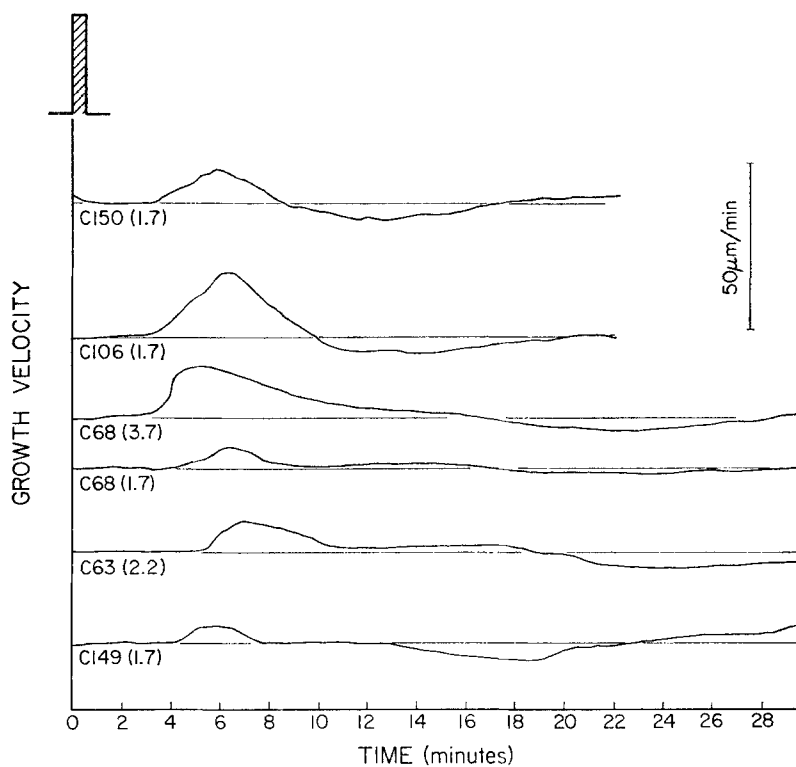


FIGURE 17. Responses of five class 2 mutants to 0.5-min pulses of broad blue light from $A = 400 \text{ pW}/\text{cm}^2$. The stimulus magnitude $\log_{10} S$ is indicated below each curve in parentheses after the number of the mutant.

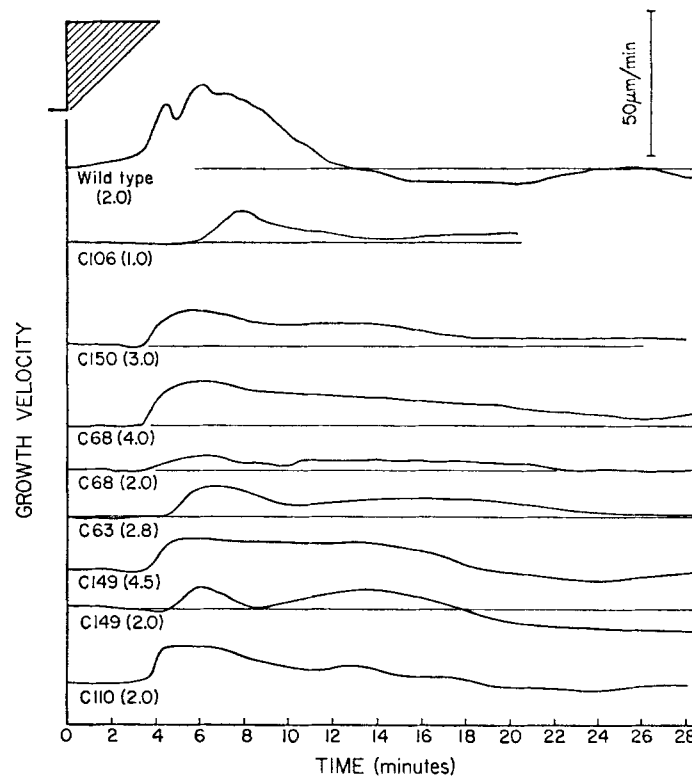


FIGURE 18. Responses of the five mutants of Fig. 17 as well as of C110 and NRRL1555 to steps of broad blue light from $A = 400 \text{ pW/cm}^2$. The step amplitude $\log_{10} (I/A)$ is indicated in parentheses after the number of each mutant.

pendence of the response magnitude on the stimulus according to a relationship of the form $R = R_0 S / (S + S_0)$ where R_0 is the size of the maximal response and S_0 is the stimulus to give a half-maximal response. For a review of diverse organisms exhibiting this dependence see Lipetz (1971) and for chemical conditions producing such a dependence see Ariens and Simonis (1964). This character is well exemplified here by both measures R_A and R_P of the growth response of *Phycomyces*. However the values of S_0 are systematically higher for R_A than for R_P . This is a reflection of the fact that with increasing stimulus size the response broadens although the maximum growth rate has saturated (possibly due to clipping of the response at some stage of the response pathway). Penn and Hagins (1972) using photovoltages from rat retinas observed the same effect.

Another striking character is the suddenness of the turn-on of the response after a long latency. The initial acceleration attains its maximum within 0.5 min after the latent period. We do not know the causes of the latent period, but we believe from its dependence on temperature that it is governed by enzymatic reactions rather than diffusion.

Systematic variations in response were observed as a function of the absolute adapted intensity for a fixed subjective stimulus. In Fig. 8, the response magnitude is seen first to increase with absolute intensity, then to peak in the "normal" range (Delbrück and Reichardt, 1956), and finally at higher intensities to drop rapidly to zero. The phototropic rate shows similar behavior. Another effect is that the bandwidth of the system increases with absolute intensity. This increase is exhibited by the decrease of latency for both pulse and sine-wave stimuli with increasing adapted intensity.

At the high intensities at which the response amplitude is diminished, there is a graded increase in the steady-state growth rate (Fig. 14) with increasing absolute intensity. This effect is half-maximal at $(1.4 \pm 0.4) \text{ mW/cm}^2$ (488 nm). Perhaps at such intensities growth-rate regulation is diminished. For crayfish reticular cells Glantz (1972) shows a similar increase in the steady-state photovoltage with intensity possibly pointing to a similarity in the mechanisms.

In response to pulse stimuli of various durations, several systematic changes occur. When the pulse duration becomes comparable to the latency and response width, then as expected there is an accompanying change of response shape. The later part of a long duration pulse has no effect on determining the latency or the initial acceleration of the response. Interestingly, for equal S the longer stimuli (5 min) produce a larger response.

A noteworthy characteristic of the growth response is the fine structure (Fig. 11) which appears to be a lightly damped (0.035 dampening ratio) oscillation superposed on a saturated response. The natural period is $1.6 \pm 0.1 \text{ min}$ and is observed to be fairly constant under different conditions. For fixed S , the amplitude of this ringing increases gradually with the level of adaptation. The oscillations probably originate in an output part of the system since it is also observed in response to "avoidance" stimuli. We may speculate that this fine structure is produced by some saturating chemical feedback loop or by second-order low-pass filtering.

In the Introduction we mentioned some of the characteristics of the light growth response that commend it for study in depth. We may now add a few more features. Responses to stimuli of different wavelengths are alike, indicating that we may have a one-pigment system. The growth velocity resembles the slow potentials recorded by neurophysiologists from the photoreceptor, horizontal, and bipolar cells of higher organisms. With the tracking machine we can display these responses much as they have. Of course our time scale is in minutes rather than milliseconds. Finally the light growth response is amenable to white-noise analysis (Marmarelis and Naka, 1972).

We are presently implementing equipment to use as an input signal white noise (a random input signal with constant power spectral density over the

system bandwidth). Both this input signal and the light growth response will be correlated on the computer to obtain a nonlinear transfer functional of the system. Because *Phycomyces* is a slowly responding system (pulse stimuli require 45-min intervals between their repetitions) white-noise techniques are particularly desirable to make the most efficient use of the available stimulus time. Using this technique we hope to characterize mutants many times faster than in the past. The present work provides the framework for the design and testing of the white-noise analysis.

Behavioral mutants are being used as a means of dissecting the stimulus-response pathway. Mutants of the stimulus-response pathway have been divided into three groups (Bergman, 1972 *a* and Results section). Class 1-1 mutants C47 and C21 have thresholds higher than that of wild type by a factor of 6×10^4 . C47 may have a reduced concentration of receptor pigment since it shows a completely normal response (Fig. 15). C21 has an abnormally delayed maximum in its response (Fig. 16) responsible for the phototropic anomalies of rapid small amplitude "hunting" and large aiming errors. Class 1-2 mutants have greatly increased "refractory periods" (inability to respond to a stimulus following a large stimulus). Class 2 (output) mutants phototrope weakly, even with 280-nm light. They do not however have a raised threshold for response. Their light growth response is prolonged and easily saturated. This results in poor troping because the response is of small amplitude and is spread out with the twisting of the cell such that there is little differential in growth rate between one side and the other.

Mutants should exist which regenerate their pigment after bleaching more slowly than does wild type. Such mutants would see normally at low intensities but not at high intensities ("bright blind"). This type of mutant has been elusive. Another class of mutants searched for are those having a receptor pigment with a reduced absorption cross section. Such mutants should be unable to see at low intensities ("night blind") but at the same time able to see at higher light intensities than wild type ("bright seeing"). Both of these types of mutants should be intimately connected to the receptor pigment complex.

Combining the precision of the tracking machine with its efficient use through white-noise analysis we hope to be able to use mutants of *Phycomyces* to probe the molecular mechanisms of its response to light.

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REFERENCES

- ARIENS, E. J., and A. M. SIMONIS. 1964. Drug-receptor interaction of one or more drugs with one receptor system. In *Molecular Pharmacology*. E. J. Ariens, editor. Academic Press, Inc. New York. 1:119.
- BARTLETT, N. R., and S. MACLEOD. 1954. Effect of flash and field luminance upon human reaction time. *J. Opt. Soc. Am.* 44:306.
- BERGMAN, K. 1972 *a*. Sensory responses of *Phycomyces*: I. Blue-light control of sporangiophore initiation. II. Classification of *mad* mutants. Ph.D. Thesis. California Institute of Technology, Pasadena.
- BERGMAN, K. 1972 *b*. Blue-light control of sporangiophore initiation in *Phycomyces*. *Planta (Berl.)*. 107:53.
- BERGMAN, K., A. P. ESLAVA, and E. CERDÁ-OLMEDO. 1973. Mutants of *Phycomyces* with abnormal phototropism. *Mol. Gen. Genet.* 123:1.
- BERGMAN, K., P. V. BURKE, E. CERDÁ-OLMEDO, C. N. DAVID, M. DELBRÜCK, K. W. FOSTER, E. W. GOODELL, M. HEISENBERG, G. MEISSNER, M. ZALOKAR, D. S. DENNISON, and W. SHROPSHIRE, JR. 1969. *Phycomyces*. *Bacteriol. Rev.* 33:99.
- BLAAUW, A. H. 1909. Die Perzeption des Lichtes. *Recl. Trav. Bot. Neerl.* 5:209.
- BLAAUW, A. H. 1914. Licht und Wachstum. I. *Z. Botan.* 6:641.
- BLAAUW, A. H. 1915. Licht und Wachstum. II. *Z. Botan.* 7:641.
- BLAAUW, A. H. 1918. Licht und Wachstum. III. (Die Erklärung des Phototropismus). *Meded. Landbouwhoges. Wageningen.* 15:89.
- CASTLE, E. S. 1930. Phototropism and the light-sensitive system of *Phycomyces*. *J. Gen. Physiol.* 13:421.
- CASTLE, E. S. 1932. Dark adaptation and the dark growth response of *Phycomyces*. *J. Gen. Physiol.* 16:75.
- CASTLE, E. S. 1966. Light responses of *Phycomyces*. *Science (Wash. D.C.)*. 154:1416.
- CASTLE, E. S., and A. J. M. HONEYMAN. 1935. The light growth response and the growth system of *Phycomyces*. *J. Gen. Physiol.* 18:385.
- CERDÁ-OLMEDO, E. 1973. *Phycomyces*. In *Handbook of Genetics*. R. C. King, editor. In press.
- CHARLTON, J. S., and K. I. NAKA. 1970. Effects of temperature change on the cat fish S-potentials. *Vision Res.* 10:1119.
- CURRY, G. M., and H. E. GRUEN. 1959. Action spectra for the positive and negative phototropism of *Phycomyces* sporangiophores. *Proc. Natl. Acad. Sci. U. S. A.* 45:797.
- DELBRÜCK, M., and W. REICHARDT. 1956. System analysis for the light growth reactions of *Phycomyces*. In *Cellular Mechanisms in Differentiation and Growth*. Dorothea Rudnick, editor. Princeton University Press, Princeton. 3-44.
- DELBRÜCK, M., and W. SHROPSHIRE, JR. 1960. Action and transmission spectra of *Phycomyces*. *Plant Physiol.* 35:194.
- FOSTER, K. W. 1972. The photoresponses of *Phycomyces*: Analysis using manual techniques and an automated machine which precisely tracks and measures growth during programmed stimuli. Ph.D. Thesis, California Institute of Technology, Pasadena.
- FOSTER, K. W. 1973. How to track sporangiophores of the fungus *Phycomyces*. *Rev. Sci. Instrum.* In press.
- GLANTZ, R. M. 1972. Visual adaptation: A case of nonlinear summation. *Vision Res.* 12:103.
- HAMILTON, W. C. 1964. *Statistics in Physical Science*. The Ronald Press Company, New York. 150.
- HAMILTON, W. C. 1964. *Statistics in Physical Science*. The Ronald Press Company, New York. 150.
- HEISENBERG, M., and E. CERDÁ-OLMEDO. 1968. Segregation of heterokaryons in the asexual cycle of *Phycomyces*. *Mol. Gen. Genet.* 102:187.
- HESS, B., and A. BOITEUX. 1971. Oscillatory phenomena in biochemistry. *Annu. Rev. Biochem.* 40:237.

- LIPETZ, L. E. 1971. The relation of physiological and psychological aspects of sensory intensity. In *Principles of Receptor Physiology*. W. R. Loewenstein, editor. Handbook of Sensory Physiology. Springer-Verlag, New York. 1:191.
- MARMARELIS, P. Z., and K. NAKA. 1972. White-noise analysis of a neuron chain: An application of the Wiener theory. *Science (Wash. D.C.)*. 175:1276.
- OORT, A. J. P. 1932. Die wiederherstellung der empfindlichkeit nach einem Lichtreiz. *K. Ned. Akad. Wet. Proc.* 34:3.
- OOTAKI, T., A. C. LIGHTY, M. DELBRÜCK, and W.-J. HSU. 1973. Complementation between mutants of *Phycomyces* deficient with respect to carotenogenesis. *Mol. Gen. Genet.* 121:57.
- PENN, R. E., and W. A. HAGINS. 1972. Kinetics of the photocurrent of retinal rods. *Biophys. J.* 12:1073.
- REICHARDT, W., and D. VARJÚ. 1958. Eine Inversionsphase der phototropischen Reaktion (Experimente an dem Pilz *Phycomyces blakesleeana*). *Z. Physik. Chem. Neue Folge.* 15:297.
- RUSHTON, W. A. H. 1961. Dark-adaptation and the regeneration of rhodopsin. *J. Physiol. (Lond.)* 156:166.